Acidification Methods for Stabilization and Storage of Salmon By-Products

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ABSTRACT. High-quality by-products from fish processing are sometimes discarded unless fishmeal plants are located nearby. Other preservation methods, such as acidification, are less commonly used to inhibit spoilage. In this study, individual salmon by-products (heads, viscera, and a mixture) were stabilized through fermentation by lactic acid bacteria and through ensilage by direct acidification. Lipid and protein quality decreased in all samples with storage time (up to 120 days). Of note, viscera and heads preserved separately consistently maintained a lower, more effective pH than when mixed together, regardless of treatment, which has major implications for how fish processing waste should be collected and stored if maximum nutritional quality is to be preserved.

KEYWORDS. By-products, silage, fermentate, salmon, heads, viscera

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INTRODUCTION

Fish processing in Alaska is responsible for producing over one million metric tons of by-products each year (Crapo and Bechtel, 2003). When fishmeal plants are located nearby, fish oils and meals can be conveniently produced from these discards. However, due to the seasonal nature of Alaska's salmon harvest (June through September) and the remote locations of the catch, salmon by-products are generally not fully utilized. Shore-based salmon processors often grind waste material and pump it into the ocean. Alternative methods of preservation are needed to decrease the loss of valuable marine proteins and oils, and to provide salmon processors with environmentally sound options for adding value to by-products currently discarded as waste.

Acidification is a well-known method for inhibiting microorganisms and promoting autolysis of animal tissues, thereby preserving perishable foods such as fish. The simplicity of the process and relatively low cost associated with acidification can be an advantage when handling small batches of fish by-products. Both organic and inorganic (mineral) acids are suitable for silage production, although a lower starting pH is required for inorganic acids to remain effective. Organic acids, while more expensive, are better able to prevent spoilage, and the silage does not require neutralization before use, unlike their inorganic counterparts (Raa and Gildberg, 1982). Formic acid silages have been most commonly studied although other acids (such as acetic, benzoic, citric, propionic, sorbic, hydrochloric, phosphoric, and sulfuric) have also been evaluated for ensiling fish by-products (Lo et al., 1993; Raa and Gildberg, 1982). The target pH of fish silage is dependent upon the type of acid chosen. For example, the pH of ground fish must be reduced to 2.0 when sulfuric acid is applied (Raa and Gildberg, 1982), whereas the necessary pH to produce formic acid silage is only 4.5 (Espe and Lied, 1999). Expensive organic acids have been evaluated in combination with less costly inorganic acids, which were applied first to lower the pH of the silage to the pK_a of the organic acid for maximum efficacy. Conditions of acidification are known to substantially change lipid and protein quality of fish during ensilage (Dapkevicius et al., 1998).

Fermentation by lactic acid bacteria (LAB) is also a well-known method for preserving food (Faid et al., 1997; Stiles, 1996; Van Wyk and Heydenrych, 1985). Spoilage of fish is accelerated by normal flora bacteria that metabolize free amino acids for energy and release ammonia as a waste product (Raa and Gildberg, 1982). LAB compete for resources by

producing lactic acid, which decreases the pH of their environment and inhibits spoilage bacteria. Other inhibitory compounds such as bacteriocins, acetic acid, and ethanol are also produced during LAB fermentation. However, since fish do not contain significant amounts of carbohydrate, a fermentable sugar must be added to support bacterial growth (Dong et al., 1993). In tropical countries, potential sources of sugar include molasses and wastes from sugar cane production. However, in colder climates such as Alaska, the purchase price of a carbohydrate source must be factored into the cost of producing a fermentate. There are several advantages to stabilizing fish by-products through fermentation rather than through direct acidification. Fish odor disappears, nutritional value is retained, and the fermented product is perceived as probiotic and therefore more wholesome (Glatman et al., 2000; Gelman et al., 2001). Starter cultures containing combinations of bacteria (*Lactobacillus plantarum*) and yeast (*Saccharomyces* sp.) have also been used to preserve and transform fish by-products into a stable ingredient suitable for animal feeds (Faid et al., 1997).

Silages and fermentates can serve as either short- or long-term methods for preserving fish by-products. However, to be cost-effective the final product must have a market. Successes have been found by drying the ensiled material into fishmeal, as well as by incorporating it into feeds (Arason et al., 1990). Short periods of ensilage result in less protein hydrolysis and longer peptide chains, which can be successfully incorporated into ruminant feeds (Arason et al., 1990). Silage undergoing a longer period of hydrolysis will contain shorter peptides more suited for use in aquaculture feeds or as additives for chicken and pig diets. Silages are a reasonable option for preserving fish by-products, especially with seasonal harvests in remote locations where it is not cost-effective to build a fishmeal facility.

Silages have been prepared from a variety of fish and fish by-products (Tocher et al., 1997; Dong et al., 1993), but direct comparisons between individually ensiled fish components, such as heads and viscera, are not available. Raw material differs among fish species and can change seasonally, in addition to the large fish to fish variation found within species. Differences are also apparent among individual fish by-products, with viscera demonstrating the most rapid rate of hydrolysis compared to other raw fish materials (Espe and Lied, 1999). However, fish by-products are typically mixed together during handling, even though some components may have adverse effects on others. The objective of this study was to compare the efficacy of different methods of acidification for stabilizing

individual by-products, such as salmon heads, viscera, and mixtures of both. Salmon by-products were placed into four different treatment groups: preservation without acidification; acidification with formic acid; fermentation using LAB; and preacidification with a lower quantity of formic acid to reduce the pH and eliminate bacterial competition prior to LAB inoculation. Increased knowledge in stabilizing by-products will enable small, remote fishing operations to maximize the value of their underutilized fish components.

MATERIALS AND METHODS

Fish By-Products

Pink salmon (*Oncorhynchus gorbuscha*) heads, viscera, and head-viscera mixtures were collected from a commercial processor in Kodiak, Alaska, in August 2006. All samples were shipped frozen to Fairbanks for processing. Salmon heads and viscera were individually homogenized (90 s, high speed for heads, 30 s for viscera) using a Waring Heavy Duty blender (Waring Products, Inc., Torrington, CT), before transferring 250 g aliquots of heads, viscera, or equal parts of a head-viscera mixture to glass containers (0.5 L). Sodium chloride (NaCl) was incorporated into all fish by-products at a concentration of 2.5% (w/w) to inhibit spoilage bacteria (Ahmed et al., 1996). Nine containers were set up for each treatment group so that storage experiments could be evaluated in triplicate at 30, 60 and 120 days without having to resample from any previously opened containers. Four treatment groups were compared: untreated salmon by-products; acidified using formic acid; fermentation with LAB; and preacidification using a lower quantity of formic acid to reduce the pH to 4.1 prior to LAB inoculation.

Direct Acidification

The acid to fish ratio was determined for salmon heads, salmon viscera, and mixtures of both by adding sufficient formic acid to reduce the pH to 3.8 in all samples. Typically, this required 1.2% formic acid addition for heads, 1.0% for head-viscera mixtures, and 0.96% for viscera samples. For preacidification studies, less formic acid was added prior to the addition of lactic acid bacteria, since many LAB are inhibited below pH 4.0 (Cai et al., 1998). Salmon heads were acidified with 0.8% formic acid, whereas viscera and head-viscera mixtures each received 0.6% formic acid.

Acidification by Lactic Acid Bacteria

A lactic acid bacteria (LAB) cocktail consisting of 5 strains was prepared for inoculation (4×10^4 CFU/ml) since different LAB strains impart different chemical properties and inhibitory characteristics (Gelman et al., 2001). Homofermentative strains (bacteria that produce only lactic acid from glucose) were chosen to maximize lactic acid production since heterofermentative LAB strains do not improve silage quality (Cai et al., 1998). Bacterial strains were: *Lactobacillus casei* (NRRL B-1922), *Lactobacillus curvatus* (NRRL B-4562), *Lactobacillus plantarum* (NRRL B-4496), *Lactococcus lactis* (NRRL B-1821), *Pediococcus pentosaceus* (NRRL B-14009). All strains were tolerant to NaCl at 2.5% and capable of fermenting sucrose. All LAB were obtained from A.P. Rooney of the USDA ARS Microbial Genomics and Bioprocessing Research Unit in Peoria, IL. Since fish tissues usually contain only small quantities (< 0.5%) of carbohydrate (Gram and Huss, 1996), a fermentable carbon source was required for lactic acid production. For this study, sucrose was added to all LAB-inoculated samples at a concentration of 15%. Additionally, all bacteria were cross-screened for bacteriocins that might prove antagonistic to the growth of other LAB strains in the inoculum. This was accomplished by streaking lines of each culture directly onto plates containing BHI agar (Oxoid, Ltd., Basingstoke, Hampshire, England). A grid pattern was established on the plates so that every strain of bacteria intersected every other strain. Any inhibition of bacterial growth observed at the point of intersection was examined further, to verify that the interaction was minor and therefore not likely to compromise bacterial growth rates when diluted within the 250 g sample of silage.

Storage of Samples

Glass containers were sealed to promote a reduced oxygen environment, thereby inhibiting the growth of mold and supporting the fermentative processes of LAB to maximize lactic acid production. Containers were held at 21°C and sampled at 30, 60, and 120-days to determine compositional analyses (moisture, ash, protein, and lipids), pH, bacterial counts (total CFU/g, lactic acid bacteria, and coliforms), and chemical analyses (lactic acid levels).

Bacterial Enumeration

Total bacterial cell counts were carried out in triplicate by plating serial dilutions from Butterfield's Buffer (Hardy Diagnostics, Santa Maria, CA)

onto BHI agar, and then incubating at 35°C for up to 48 h. Coliforms were similarly enumerated on MacConkey agar (Oxoid, Ltd., Basingstoke, Hampshire, England), and presumptive lactic acid bacteria were counted on MRS agar (Oxoid, Ltd., Basingstoke, Hampshire, England). BHI plates were allowed to incubate up to 1-week (35°C) to check for growth of mold.

Lactic Acid Assay

Lactic acid assay was performed according to the procedure of Taylor (1996), where hot sulfuric acid was used to cleave acetaldehyde from lactic acid molecules. The acetaldehyde reacts with copper and p-phenylphenol to produce a chromogen, which was read spectrophotometrically at 570 nm using a SpectraMax Plus microplate spectrophotometer (Molecular Devices, Union City, CA). All samples were run in triplicate and were diluted to fall within the standard graph range of 10^{-2} to 10^{-3} g/L. The assay was linear to a sensitivity of 0.4 g/L.

Compositional Analyses

Three samples from each treatment method were analyzed in duplicate for moisture, protein, lipids, and ash. Moisture was determined gravimetrically by drying samples at 103°C for 24 h and measuring water loss (method 952.08, AOAC, 1990). Protein was measured by drying samples (103°C, 24 h) and analyzing for nitrogen content on an Elementar Rapid NIII analyzer (Mt. Laurel, NJ) using WINRAPID™ software to calculate protein values. Lipids were determined by processing dried samples on a Soxtec Model 2043 (Foss Analytical, Denmark) using a methylene dichloride extraction solvent, after which lipid-rich solutions were evaporated to dryness to remove solvent and then weighed. Ash content was determined by placing samples into a muffle furnace at 550°C for 24 h and then weighing the remaining material (method 938.08, AOAC 1990). As the silage hydrolyzed and became more liquid over 120 days of storage, most samples separated into three or more layers depending on the treatment. Sampling protocols involved complete mixing (manually) of the contents of each container prior to sample removal. Then, each sample was centrifuged (5,000 g; 10-min) to allow separate layers to be tested for their individual compositions. The aqueous fraction was designated as the layer containing soluble proteins, and quantification of nitrogen was performed using the Elementar Rapid NIII analyzer described above.

Statistical Analysis

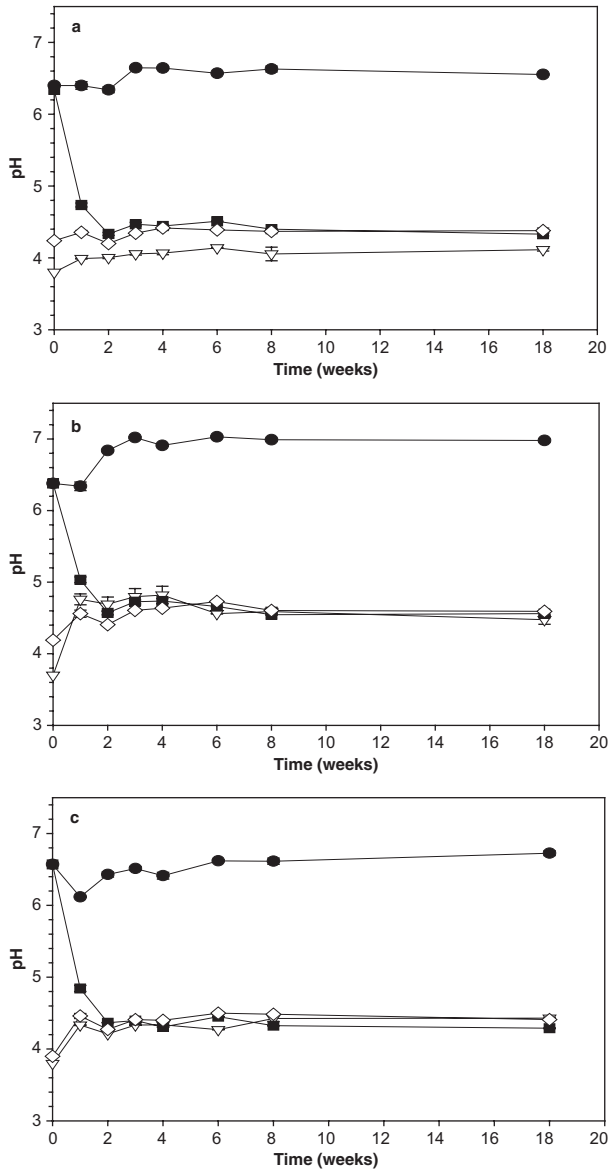
The effects of all treatments and by-product types (i.e., heads, viscera, or mixed) were investigated using one way analysis of variance (ANOVA) conducted with the Statistica v 7.1 software package (Statsoft, Tulsa, OK). The ANOVA *p*-value was set to .05, and differences between treatments were examined using the post-hoc test Tukey's unequal N honest significant differences ($p < .05$). Statistical analyses were performed for pH and composition (moisture, ash, lipid, and protein) measurements.

RESULTS AND DISCUSSION

Acidification

For this study, salmon by-products were stabilized using direct acidification, fermentation by lactic acid bacteria, and combinations of both techniques to lower the pH of the samples below the point at which spoilage bacteria normally thrive. Prior to acidification treatment, pH differences were statistically significant ($p < .05$) for heads (pH 6.77), viscera (pH 6.34), and mixtures of the two (pH 6.67). During 120 days of storage, the pH values for untreated controls remained fairly constant in all by-product samples tested (viscera, heads, and mixed), starting out near pH 6.4 and rising as high as pH 7.0 (Figure 1). When by-products were ensiled using formic acid, the pH remained relatively stable for the viscera samples at approximately pH 4, although the heads and mixed samples showed more variation with values increasing to approximately pH 4.5. When by-products were acidified with lactic acid through LAB fermentation, the pH decreased from initial values near 6.4 before stabilizing to near pH 4.5 after one week. Typically, LAB fermentations are conducted at temperatures higher than 30°C, which encourages bacterial growth and leads to increased acid production (< pH 4.0) within a few days. However, other investigators have determined that the final pH in formic-acid silages prepared from herring, cod, mackerel, and pollock was not affected by storage temperature (Espe and Lied, 1999). In this study, all samples were held at room temperature (20°C), thereby requiring more time for the LAB fermentates to decrease below pH 4. To accelerate the process, a preacidification study was initiated. A lower concentration of formic acid was incorporated into by-product samples to rapidly reduce the pH before inoculating with LAB. The result was an immediate

FIGURE 1. Change in pH over 120 days for (a) Viscera, (b) Mixed, and (c) Heads of Pink Salmon preserved without acidification (●), acidified using formic acid (▽), fermented using Lactic Acid Bacteria (■), and pre-acidified with formic acid before LAB addition (◇).

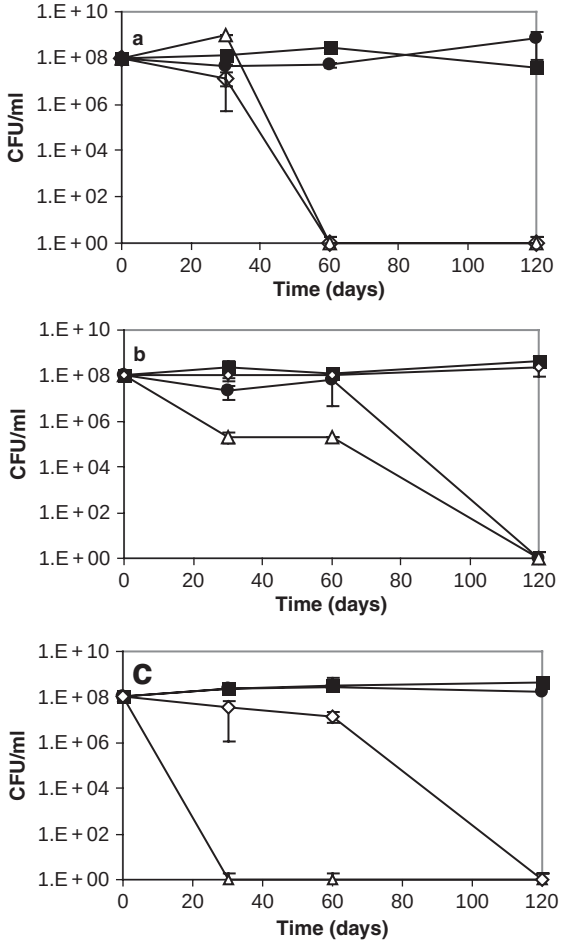


stabilization near pH 4.3. One of the treatment groups, mixed salmon by-products, consistently stabilized at a pH that was higher than the pH values for either viscera or heads alone. This suggests that processors may benefit by separating the higher pH fish heads from the lower pH viscera components, since the importance of maintaining a low pH is so critical during acidification. Silages prepared using formic acid are not subject to deterioration when the pH remains below 4.5 during storage (Espe and Lied, 1999). Additionally, by maintaining a low pH, formation of biogenic amines can be inhibited, thereby preserving the integrity of their corresponding amino acids (Espe and Lied, 1999).

Bacterial Enumeration

The quantity of bacteria in untreated samples of salmon-viscera and salmon-head by-products remained high with both initial and 120-day counts of approximately 10^8 CFU/ml of mixed growth (Figure 2). However, the untreated viscera-head mixtures decreased to zero CFU/ml during the same time interval. For all samples treated with formic acid (no LAB), significant decreases in bacterial numbers were observed, with salmon heads having no culturable bacteria at day 30, and salmon viscera displaying no growth at day 60. However, the mixed by-products with formic acid (no LAB) required 120 days to reduce the bacterial population to zero CFU/ml. All by-products fermented by LAB (without formic acid) retained colony counts ranging from 10^7 to 10^8 CFU/ml during the 120-day study. Based on Gram stains, growth on MRS agar, and lack of growth on MacConkey's agar, all bacteria were classified as presumptive LAB, with no coliforms present at day 120 in any treatment group (viscera, heads, and mixed). Preacidified by-products, which were inoculated with LAB, eventually decreased bacteria levels to zero in all samples tested with the exception of mixed by-products. It is interesting that the formic acid appeared to be less lethal in mixed silage, requiring longer time to inhibit bacterial populations than in either viscera or heads. The LAB in the preacidified mixed by-product samples also withstood the inhibitory effects of formic acid more successfully than their head and viscera counterparts, suggesting the existence of a protective effect for LAB, but not aerobic bacteria. The decreased sample acidity (higher pH) observed in mixed by-products (Figure 1) may have contributed to this difference. Alternately, since all treatment groups were stored in sealed containers, it is possible that the reactions, which occurred as the mixed by-products decomposed, depleted the oxygen more rapidly than either the heads or

FIGURE 2. Enumeration of bacteria (CFU/ml) in (a) Viscera, (b) Mixed, and (c) Heads of Pink Salmon preserved without acidification (●), acidified using formic acid (△), fermented using Lactic Acid Bacteria (■), and pre-acidified with formic acid before LAB addition (◇). Error bars represent Standard Error (n = 3).



viscera treatment groups. This would result in decreased growth of aerobic spoilage bacteria, whereas the microaerophilic lactic acid bacteria would not have been affected by low oxygen conditions. Another possibility involves the existence of an antimicrobial compound that is produced by

visceral enzymes and acts on muscle and neural tissues in the heads. Unfortunately, isolating potentially inhibitory fractions was outside the scope of this project. All acidification treatments (addition of formic acid and fermentation by lactic acid bacteria) inhibited growth of coliforms and mold in all three by-products sample types.

Lactic Acid Concentrations

Lactic acid levels were measured to confirm bacterial cell counts in inoculated samples. Since LAB are generally normal flora of fish (Gram and Huss, 1996), the presence of lactic acid in untreated controls was expected. By-product samples inoculated with LAB displayed an increase in lactic acid levels over 120 days (Figure 3a), which corresponded with an increase in the LAB cell counts graphed in Figure 2. When by-products were preacidified with formic acid, the decrease in lactic acid levels (Figure 3b) also generally corresponded to the reduced cell counts shown in Figure 2. Viscera samples inoculated with LAB consistently produced more lactic acid over 120 days than either salmon heads or mixed by-products. Uninoculated control samples generally displayed lactic acid levels below 4g/L, suggesting that lactic acid bacteria were not abundant. The concentration of lactic acid is considered a good indicator of fermentation stability of silage (Madrid et al., 1999).

Changes in Silage Composition

Moisture concentrations for salmon by-products during 120 days of storage are shown in Table 1. These values reflect the original composition of each sample (liquid viscera or more solid heads), as well as the compounds added during initial experimental set-up (formic acid, sucrose, and sodium chloride). For example, all samples inoculated with LAB received sucrose to provide a carbohydrate source for the bacteria, resulting in more volume and lower moisture content than other samples. Changes in moisture content over 120 days generally were not statistically significant for untreated samples and those directly acidified with formic acid. However, samples containing LAB experienced significant decreases in moisture, regardless of whether formic acid was added as a preacidification step, although the differences in moisture among viscera, heads, and mixed sample types were not significant after 120 days. Moisture levels are critical when evaluating the shelf life of a perishable product such as fish, since low water activities prevent microbial growth and influence many chemical changes that occur during storage (Kilinc et al., 2006).

FIGURE 3. Lactic acid levels in salmon by-products: (a) after inoculation with lactic acid bacteria in viscera (○), heads (□), and mixed viscera-head samples (▽), shown together with uninoculated controls, (viscera [●], heads [■], and mixed samples [▼]); and (b) after pre-acidification with formic acid in viscera (○), heads (□), and mixed viscera and heads (▽), shown together with uninoculated, formic-acidified controls, (viscera [●], heads [■], and mixed viscera and heads [▼]).

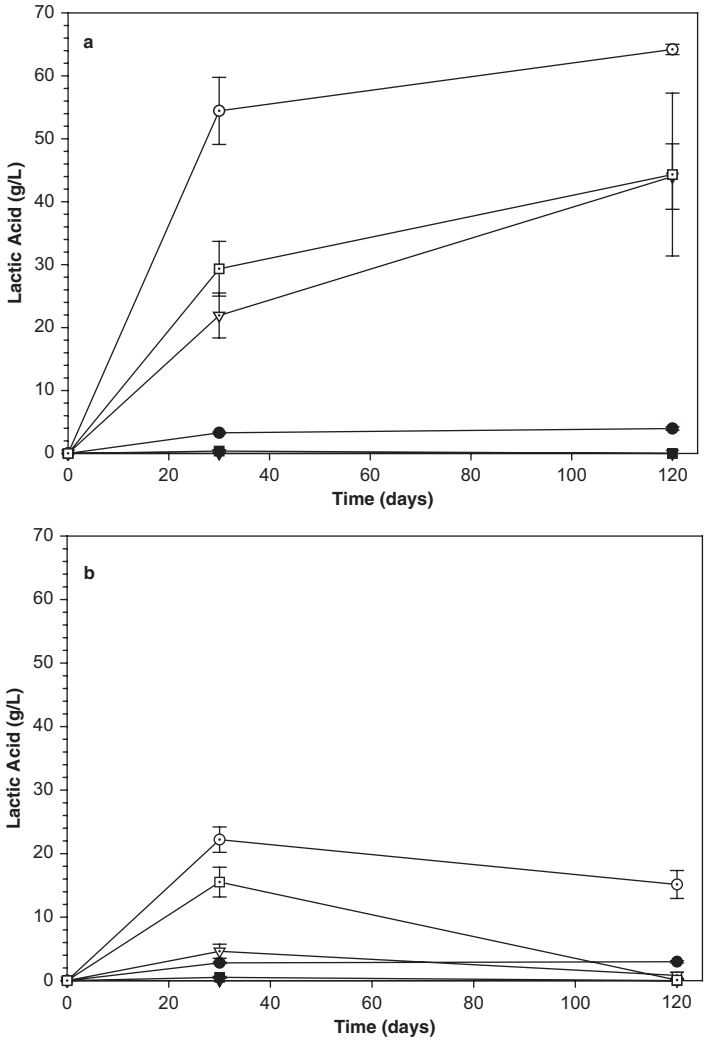


TABLE 1. Percent moisture in salmon by-products (viscera, heads, and mixed) over 120 days when subjected to different preservation methods

	Untreated		Formic Acid		LAB		Pre-Acidified LAB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viscera								
0	80.5^{bc}	0.2	80.5^{bc}	0.2	80.5^{bc}	0.2	80.5^{bc}	0.2
30	83.1^{ab}	0.1	80.9^{bc}	0.0	75.2^{def}	0.0	74.7^{ef}	0.1
60	80.2^{bc}	0.0	79.7^{bcd}	0.1	73.0^{fg}	0.1	nd	
120	79.6^{bcd}	0.1	79.1^{bcd}	0.1	68.7^{gh}	0.4	68.4^{gh}	0.1
Mixed								
0	78.1^{bcd}	0.5	78.1^{bcd}	0.5	78.1^{bcd}	0.5	78.1^{bcd}	0.5
30	82.1^{ab}	0.1	78.5^{bcd}	0.1	72.0^{fg}	1.8	74.7^{ef}	0.1
60	82.1^{ab}	0.2	77.0^{cde}	0.2	70.9^{gh}	0.2	nd	
120	84.1^{ab}	0.2	77.2^{cde}	0.1	67.0^{h}	0.1	68.3^{gh}	0.3
Heads								
0	74.0^{ef}	0.8	74.0^{ef}	0.8	74.0^{ef}	0.8	74.0^{ef}	0.8
30	80.0^{bc}	0.7	77.9^{cde}	0.2	71.6^{fg}	0.1	69.8^{gh}	0.1
60	79.9^{bc}	0.0	76.4^{de}	0.3	68.7^{gh}	0.4	nd	
120	79.7^{bcd}	2.0	75.0^{ef}	0.0	65.9^{h}	0.2	65.4^{h}	0.3

Superscripted letters represent significant differences ($p < .05$) within the whole table, not just by column or row.
nd = not determined.

The percentages of ash for each sample are listed in Table 2. The initial day 0 samples were analyzed prior to the addition of 2.5% salt (NaCl), which was included to decrease the bacterial load of spoilage organisms. This is reflected in the higher ash values found at later sampling times. Other apparent fluctuations in ash content observed in samples over 120 days were likely due to shifts in the relative percentages of other components (moisture, lipids, and proteins) which decreased within the system during storage. Predictably, ash was higher in samples containing salmon heads due to the high content of bone. Preacidification had no effect on ash content of LAB-containing samples. However, by-products directly acidified with formic acid generally registered lower ash levels than either control or LAB-inoculated samples. Knowledge of ash content is important if these preserved fish products are destined for agriculture or aquaculture feeds. The mineral content (e.g., calcium, phosphorus, and magnesium) within fish bones varies significantly among different species of fish (Toppe et al., 2007). Although ash may provide a good source of calcium

TABLE 2. Percent ash in salmon by-products (viscera, heads, and mixed) over 120 days when subjected to different preservation methods

	Untreated		Formic Acid		LAB		Pre-Acidified LAB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viscera								
0	1.3^{a}	0.0	1.3^{a}	0.0	1.3^{a}	0.0	1.3^{a}	0.0
30	3.7^{de}	0.0	1.8^{ab}	0.0	3.1^{bcd}	0.0	3.2^{bcd}	0.0
60	3.8^{de}	0.1	1.8^{ab}	0.0	3.1^{bcd}	0.0	nd	
120	3.4^{d}	0.1	1.7^{ab}	0.0	3.2^{cd}	0.0	3.2^{cd}	0.0
Mixed								
0	2.1^{abc}	0.1	2.1^{abc}	0.1	2.1^{abc}	0.1	2.1^{abc}	0.1
30	4.7^{efc}	0.1	2.2^{abc}	0.1	4.0^{def}	0.0	4.0^{def}	0.0
60	4.9^{fg}	0.0	3.3^{d}	0.2	4.3^{def}	0.1	nd	
120	4.1^{def}	0.2	1.8^{ab}	0.1	4.0^{def}	0.0	3.9^{def}	0.1
Head								
0	3.5^{de}	0.1	3.5^{de}	0.1	3.5^{def}	0.1	3.5^{def}	0.1
30	4.9^{efg}	0.5	2.7^{bcd}	0.1	4.8^{efg}	0.1	4.9^{efg}	0.1
60	5.7^{g}	0.1	4.6^{efg}	0.5	1.5^{a}	0.1	nd	
120	5.2^{fg}	0.8	2.1^{ab}	0.0	4.7^{efg}	0.1	4.6^{efg}	0.1

Superscripted letters represent significant differences ($p < .05$) within the whole table, not just by column or row.
nd = not determined.

and phosphorus in the diet, it can also lead to mineral imbalances, adversely affecting animal growth and performance (Chien et al., 2005).

The initial lipid content varied significantly among different salmon by-products, with heads containing a higher percentage of lipids (7.8%) than viscera (1.3%). However, when lipid levels for untreated heads and viscera were measured over 120 days, the percentages eventually decreased to values that were not statistically significant between by-products (Table 3). Similarly, no significant differences were detected among formic acid and LAB treatments after 120 days of storage. At 30 days, addition of formic acid appeared to delay the loss of lipid in salmon-head silage but not viscera, as compared to the untreated control. This effect was not noted when LAB were present. The initial increased numerical values in lipid content observed at day 30 in formic acid treatments were unexpected, but have been previously documented in silages produced from herring, cod, and pollock during 48 days of storage (Espe and Lied, 1999) and blue whiting after 15 days (Dapkevičius et al., 1998). The importance of lipid content

TABLE 3. Percent lipid in salmon by-products (viscera, heads, and mixed) over 120 days when subjected to different preservation methods

	Untreated		Formic Acid		LAB		Pre-Acidified LAB	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Viscera								
0	1.32^{bc}	0.03	1.32^{bc}	0.03	1.32^{bc}	0.03	1.32^{bc}	0.03
30	2.77^{d}	0.12	1.71^{cd}	0.13	0.47^{abc}	0.13	0.43^{ab}	0.12
120	0.42^{ab}	0.01	0.25^{ab}	0.00	0.06^{ab}	0.01	0.11^{ab}	0.01
Mixed								
0	6.79^{ef}	0.15	6.79^{ef}	0.15	6.79^{ef}	0.15	6.79^{ef}	0.15
30	5.59^{e}	0.07	5.64^{e}	0.00	2.88^{d}	0.15	2.93^{d}	0.06
120	0.28^{ab}	0.28	0.01^{a}	0.00	0.17^{ab}	0.01	0.16^{ab}	0.01
Heads								
0	7.79^{fg}	0.19	7.79^{fg}	0.19	7.79^{fg}	0.19	7.79^{fg}	0.19
30	6.72^{ef}	1.05	8.09^{g}	0.22	6.30^{e}	0.13	5.76^{e}	0.04
120	1.18^{abc}	0.03	1.27^{bc}	0.05	0.53^{abc}	0.01	0.73^{abc}	0.01

Superscripted letters represent significant differences ($p < .05$) within the whole table, not just by column or row.

when evaluating raw materials for silage generally applies to warm-water fish, where increased lipid may slow diffusion of endogenous enzymes, thereby limiting a rapid autolysis process (Raa and Gildberg, 1982). However, cold-water fish such as salmon have more fluid lipids with lower melting points, avoiding the problem of enzyme diffusion.

All salmon by-products separated into layers during storage, regardless of treatment type. After 120 days, all samples were centrifuged to evaluate differences in partitioning among the salmon components within each of the three treatment groups (Untreated, Formic Acid, and LAB). As shown in Table 3, lipid levels of all 120-day silage treatments were low. After centrifugation, lipid content differed within layers of salmon heads and viscera, with more lipids partitioning into the top layer for heads, and bottom layer for viscera (Table 4). This may have been caused by differential binding of lipids to heavier compounds found in the viscera, but not in the heads. The only exception was found in LAB viscera fermentates, which had an overall lower quantity of lipids than other treatment groups. Protein percentages were generally higher in the bottom layer of each sample, possibly reflecting particulate protein. For samples containing heads, the ash content was also higher in the

TABLE 4. The distribution of salmon components after centrifugation of 120-day samples

	Untreated		Formic Acid		LAB	
	Mean*	SE	Mean*	SE	Mean*	SE
VISCERA						
Top layer						
moisture	82.79^{ef}	0.14	79.52^{ef}	0.37	62.23^{bc}	0.44
ash	3.36^{ab}	0.11	1.42^{a}	0.11	2.70^{ab}	0.13
lipid	0.63^{ab}	0.15	0.79^{ab}	0.24	1.69^{ab}	0.22
protein**	13.60^{cd}	0.24	15.38^{d}	0.03	17.34^{de}	0.47
Middle layer						
moisture	84.01^{ef}	0.18	82.10^{ef}	0.31	73.93^{de}	0.21
ash	3.67^{ab}	0.01	1.41^{a}	0.28	3.28^{ab}	0.01
lipid	0.09^{ab}	0.00	0.04^{ab}	0.00	0.01^{ab}	0.00
protein**	13.27^{cd}	0.21	14.68^{d}	0.15	13.14^{cd}	0.19
Bottom layer						
moisture	74.89^{de}	0.20	72.24^{de}	0.54	65.90^{bc}	0.65
ash	3.34^{ab}	0.02	2.45^{ab}	0.28	nd	
lipid	2.14^{ab}	0.02	2.85^{b}	0.16	1.08^{ab}	0.35
protein**	22.82^{f}	0.37	22.22^{f}	0.48	18.05e	0.69
MIXED						
Top layer						
moisture	66.53^{bc}	1.51	55.10^{a}	1.31	55.71^{ab}	0.42
ash	2.93^{ab}	0.05	1.95^{ab}	0.66	3.04^{ab}	0.02
lipid	9.87^{d}	1.13	20.83^{g}	1.56	9.39^{cd}	0.87
protein**	14.10^{d}	0.18	13.32^{cd}	0.12	14.58^{d}	0.12
Middle layer						
moisture	85.13^{f}	1.54	81.85^{ef}	0.04	70.93^{cd}	0.23
ash	3.65^{ab}	0.03	1.79^{a}	0.05	4.02^{abc}	0.02
lipid	0.39^{ab}	0.10	0.15^{ab}	0.01	0.04^{ab}	0.00
protein**	10.76^{bc}	0.43	13.50^{cd}	0.17	11.13^{bc}	0.08
Bottom layer						
moisture	78.72^{ef}	0.71	71.60^{cd}	0.59	62.73^{bc}	0.80
ash	6.43^{cde}	0.07	4.26^{abc}	0.67	5.59^{bcd}	0.65
lipid	1.22^{ab}	0.13	5.00^{bc}	0.89	1.40^{ab}	0.21
protein**	18.26^{e}	0.61	16.10^{de}	1.07	16.48^{de}	0.34
HEADS						
Top layer						
moisture	60.80^{b}	2.25	52.33^{a}	0.81	56.07^{ab}	0.99
ash	3.13^{ab}	0.07	1.20^{a}	0.01	3.34^{ab}	0.07

(*Continued*)

TABLE 4. (*Continued*)

	Untreated		Formic Acid		LAB	
	Mean*	SE	Mean*	SE	Mean*	SE
lipid	16.72^{f}	0.47	13.12^{e}	0.47	6.86^{cd}	0.79
protein**	8.90^{ab}	0.06	10.85^{bc}	0.35	9.99^{ab}	0.52
Middle layer						
moisture	86.50^{f}	1.90	82.46^{ef}	0.26	72.54^{de}	0.04
ash	3.70^{ab}	0.06	2.05^{ab}	0.02	4.41^{bc}	0.03
lipid	0.41^{ab}	0.06	0.15^{ab}	0.02	0.07^{ab}	0.03
protein**	8.67^{ab}	0.42	11.48^{bc}	0.02	7.92^{a}	0.04
Bottom layer						
moisture	77.30^{de}	0.51	67.47^{cd}	1.71	61.79^{b}	1.57
ash	7.20^{de}	0.89	8.29	1.60	6.88^{cde}	0.90
lipid	1.42^{ab}	0.16	2.56^{ab}	0.53	1.40^{ab}	0.05
protein**	15.95^{de}	0.90	15.10^{d}	0.48	14.14^{d}	0.62

*Letters represent significant differences ($p < 0.05$) within the four categories (moisture, ash, lipid, and protein) throughout the table.

**Protein and other nitrogenous compounds.

***Some layer compositions may not add up to 100% due to the presence of carbohydrates, which were not measured.

bottom layer, presumably due to an increased concentration of bone. Knowing the compositional content of the separating layers may prove useful to silage producers who wish to provide specialized products, such as high- or low-ash silages.

During ensilage, proteins break down into smaller peptide units, amino acids, and other compounds resulting in an overall increase in protein solubility. Assays that measure nitrogen can be used to directly follow changes in proteins that occur over time. Distribution of nitrogen varied among samples after 120 days (Table 5), with some significant differences observed among by-product types. For example, lactic acid fermentates prepared with viscera were lower in nitrogen content than fermentates composed entirely of heads, although this effect was only observed in the bottom layers where protein had previously been found to partition (Table 4). Significant differences were also noted among treatments, with LAB-fermented viscera containing higher percentages of nitrogen in the top and bottom layers than either the formic acid silage or untreated control. Efficient utilization of dietary protein is important for survival and growth of fish. Protein quality is especially critical when formulating aquaculture feeds

TABLE 5. Distribution of nitrogen (%) in centrifuged layers after 120 days

	Viscera		Mixed		Heads	
	Mean	SE	Mean	SE	Mean	SE
Untreated						
top layer	27.37^{abc}	0.16	32.70^{cde}	0.38	26.57^{abc}	0.50
middle layer	26.71^{abc}	0.29	24.96^{abc}	0.90	25.90^{abc}	1.62
bottom layer	45.92^{fg}	0.13	42.34^{ef}	1.24	47.52^{g}	2.06
Formic Acid						
top layer	29.42^{bc}	0.34	31.08^{bd}	0.97	28.98^{bc}	0.98
middle layer	28.08^{bc}	0.21	31.48^{cde}	0.72	30.68^{bcd}	0.28
bottom layer	42.50^{fg}	0.46	37.43^{e}	1.64	40.33^{ef}	1.12
Lactic Acid Bacteria						
top layer	35.74^{de}	0.59	34.56^{de}	0.40	31.17^{bcd}	1.70
middle layer	27.08^{abc}	0.31	26.38^{abc}	0.28	24.73^{abc}	0.29
bottom layer	37.18^{e}	0.82	39.05^{ef}	0.66	44.10^{fg}	1.71
Pre-Acidified LAB						
top layer	36.11^{de}	0.16	33.43^{cde}	0.27	33.81^{cde}	0.78
middle layer	30.34^{bcd}	0.30	26.71^{abc}	0.76	22.92^{a}	0.25
bottom layer	33.55^{cde}	0.26	39.86^{ef}	1.02	43.27^{fg}	1.03

Superscripted letters represent significant differences ($p < .05$) within the whole table, not just by column or row.

since fish metabolize protein preferentially to lipids or carbohydrates as an energy source (Peres and Oliva-Telesa, 2007).

CONCLUSION

Knowledge of the effects of individual fish components on the resultant silage is important for producing the best quality, most stable commodity for agriculture and aquaculture feeds. In this study, salmon by-products (heads, viscera, and a mixture) were stabilized through fermentation by lactic acid bacteria and through ensilage by direct acidification with formic acid. Stable silage was produced from all treatment groups after 120 days. Addition of LAB was very effective in reducing the pH to approximately 4.5, whereas the formic acid treatment was initially at pH 3.8 but increased to 4 or greater within a week. Viscera and heads that were preserved separately for 120 days maintained a slightly lower pH than those that were mixed together. The composition of silages made

from individual by-products was very different on day 0, with heads having lipid levels of 7.8% and viscera 1.3%; however, after 120 days, most of the extractable lipid was absent. Samples from all treatment groups had liquefied by 120 days of storage and still contained significant amounts of nitrogen in all fractions. These findings have major implications for how fish by-products should be stored prior to processing to maximize the quality of silage.

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